DIURNAL ACID METABOLISM IN VERNAL POOL *ISOETES* (ISOETACEAE)

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ABSTRACT

Leaves of the vernal pool species *Isoetes howellii* and *I. orcuttii* possess a Crassulacean Acid Metabolism-like pathway while submerged. It is proposed that this was selected because CO_2 availability is greatly reduced during the day in these pools, putting a premium on CO_2-fixation at night. Diurnal changes in physical and chemical characteristics in two vernal pools are compared with diurnal changes in malic acid concentration in *Isoetes* leaves. The data are consistent with the hypothesis.

Isoetes howellii Engelmann (Isoetaceae) is distributed throughout California and other western states in more-or-less temporary vernal pools (Munz, 1959). Because of the mediterranean climate, vernal pools are filled by winter rains and dry out during the summer drought. *Isoetes howellii* prefers deep (20–30 cm) pools (Kopecko and Lathrop, 1975). As a result, it remains submerged longer than most other vernal pool species and is entirely absent from very shallow pools (Zedler and Ebert, 1979). Vernal pools are heavily vegetated and often *I. howellii* will grow in very dense more or less monotypic stands (e.g., Purer, 1939) noted 4000–6000 plants/m^2 in some pools on mesas near San Diego). By late spring or early summer all species are emergent and as the water table drops most die. Pfeiffer (1922) stated that *Isoetes* corms can survive drought, but she did not mention the species on which she based this conclusion.

Recent evidence has been presented that *Isoetes howellii* has a diurnal acidification/deacidification cycle similar to Crassulacean Acid Metabolism (Keeley, 1981). Submerged leaves of *I. howellii* fix CO_2 at night into malic acid and this product accumulates through the night. During the day malic acid is broken down, resulting in a diurnal fluctuation of 100–200 μequivalents titratable acidity per gram fresh weight. The major difference between *Isoetes howellii* and terrestrial CAM plants lies in stomatal behavior. The prototype CAM plant closes stomates during the day thus restricting CO_2 uptake to the night. Sculthorpe (1967) contends that stomata on submersed aquatics are functionless because of the occlusion of the stomatal apertures by cuticular waxes. Submersed *Isoetes howellii* apparently is no exception as evidenced by the thick wax which lines the stomatal aperture (D. B. Walker, UCLA, pers. comm., 1979). *Isoetes howellii* is capable of CO_2-uptake in both the light and the dark.

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The functional significance of CAM-like diurnal acid metabolism in an aquatic plant is unknown. In terrestrial plants Crassulacean Acid Metabolism was selected as a means of increasing water-use efficiency (Kluge and Ting, 1978). This arises from the daytime decarboxylation of malic acid producing an endogenous CO_2 source which maintains C_3-type photosynthesis while stomates are closed. Diurnal acid metabolism in submerged leaves of *Isoetes howellii* undoubtedly is not related to water-use efficiency. However, daytime generation of an internal CO_2 source could have been selected for other reasons. For example, if CO_2 availability were greater at night than during the day CAM could be adaptive in an aquatic environment.

Daytime reduction of CO_2-availability is possible even in pools where the total inorganic carbon is greater than in air. In water, free-CO_2 predominates below pH 6 but is gradually replaced by HCO_3^- and CO_3^{2-} at higher pH. Free CO_2 is the "preferred" substrate for photosynthesis by terrestrial and aquatic plants (Raven, 1970). As free CO_2 is consumed the pH in unbuffered pools rises (Hutchinson, 1957). Above pH 8 free CO_2 is replaced by HCO_3^- and CO_3^{2-}. Although some aquatic plants can take up HCO_3^-, many can not. Preliminary evidence indicates *I. howellii* uses HCO_3^- poorly, if at all. A comparison of C^{14}-fixation (at constant inorganic carbon levels) in the light at pH 5 and pH 8 showed an 80% reduction in C^{14}-fixation at the higher pH (Keeley, unpublished data).

I hypothesize that one selective advantage of "Crassulacean Acid Metabolism" in *Isoetes howellii* is that this pathway provides an internal CO_2 source during the day when externally derived CO_2 becomes limiting to C_3 photosynthesis. The purpose of this study was to compare diurnal changes in malic acid in *Isoetes* leaves with diurnal changes in CO_2 concentration in the pools. *Isoetes orcuttii* A. Eaton is the only other species in the genus commonly found in vernal pools. It is a California endemic, often found in close association with *I. howellii* (Kopecko and Lathrop, 1975), and it was included in this study.

Methods

Two pools were studied, one in early April 1979 on Miramar Naval Air Station, San Diego Co. and the other in late April 1980 on Mesa de Colorado, Santa Rosa Plateau, Riverside Co. At 0600 hr and 1800 hr (±30 min) several physical and chemical parameters of the pools were measured and submerged leaf samples were collected from *I. howellii* at the Miramar site and from *I. howellii* and *I. orcuttii* at the Mesa de Colorado site.

Pool parameters were measured as follows: temperature and oxygen concentration with a YSI oxygen meter, pH with a Markson pH meter, and carbon dioxide concentration titrametrically as described by

TABLE 1. DIURNAL CHANGES IN PHYSICAL AND CHEMICAL PARAMETERS OF TWO VERNAL POOLS AND MALIC ACID CONCENTRATION OF *Isoetes* LEAVES SUBMERGED IN THOSE POOLS.

	Pool parameters					Malic acid (mg/gm FW) in leaves			
						I. howellii		*I. orcuttii*	
PST hr	C°	O_2 ppm	pH	Free CO_2 (mg/l)	Total CO_2 (mg/l)	$\bar{x} \pm$ S.D.	*N*	$\bar{x} \pm$ S.D.	*N*
	Miramar 8 Apr 1979								
0600	16.0	2.1	6.8	7.5	38.7	7.1 ± 1.5	6	—	—
1800	22.9	12.0	9.0	0	27.2	1.8 ± 0.4	6	—	—
	Mesa de Colorado 26 Apr 1979								
0600	12.5	7.4	6.3	9.5	24.3	12.5 ± 2.8	2	13.1 ± 2.6	2
1800	21.0	11.0	8.5	0	10.6	3.3 ± 0.2	2	3.7 ± 1.4	2

Golterman *et al.* (1978). *Isoetes* leaves were washed in distilled water, blotted dry, weighed, ground in a blender with distilled water, filtered through cheesecloth, and centrifuged at low speed. The supernatant was deproteinized with 1.0 N perchloric acid placed on ice and returned to the lab and assayed for malic acid with an enzymatic end-product assay (Bergmeyer, 1974, pp. 1585–1589).

Isoetes howellii and substrate were transplanted to artificial outdoor pools on campus so they could be sampled every 6 hrs over a two-day period.

RESULTS AND DISCUSSION

Table 1 shows the diurnal changes in physical and chemical parameters of the two vernal pools and malic acid levels in *Isoetes* leaves. During the day the temperature in the pools rose, and associated with this was an increase in oxygen, presumably due to algae and macrophyte photosynthesis. This likely accounts for the decreased free-CO_2 concentration, which in turn would cause the pH of the water to rise (Hutchinson, 1957). Evening pH values of 8.5 and 9.0, coupled with daytime total-CO_2 losses greater than the free-CO_2 losses, suggest that some species in the pools may be using HCO_3^- to some extent. Both *Isoetes howellii* and *I. orcuttii* have substantial malic acid pools in their leaves in the morning, and these are greatly depleted by the end of the day.

Some of these parameters were followed more closely over a time course of two days in artificial pools in May 1979 (Fig. 1). The fluctuation patterns of free-CO_2 in the pool (as indicated by pH) and malic acid in the leaves are reversed. Free-CO_2 in the water was largely depleted by noon and the bulk of the malic acid in the leaves was

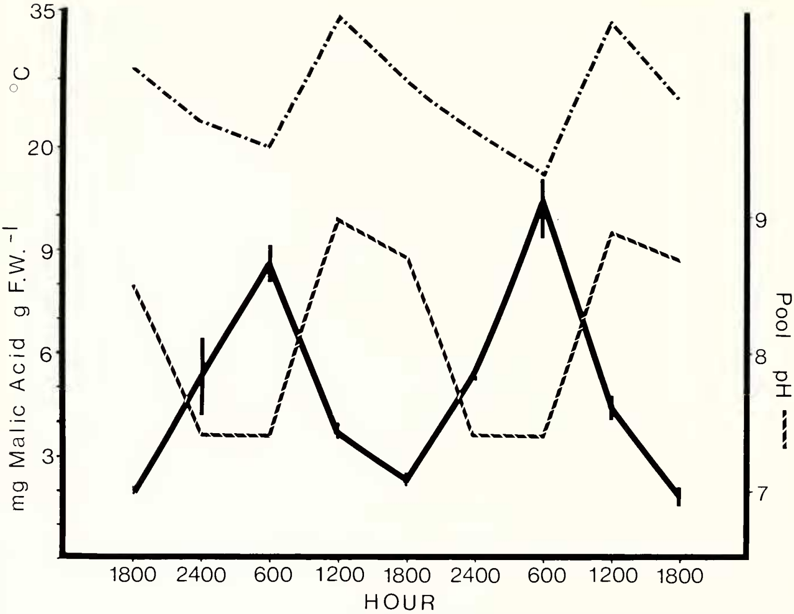

FIG. 1. Diurnal fluctuations in pool temperature and pool pH (dashed lines) in artificial pools and malic acid concentration in submerged *Isoetes howellii* leaves. Each point is the mean of two replicates, vertical bars represent ±1 S.D.

broken down by this time. Between 1800 hr and 2400 hr the pool pH dropped to a range where free-CO_2 was once again present. Through the night the pH did not drop below 7.3, indicating that free-CO_2 did not account for much more than 10 percent of the total CO_2 in the water. Malic acid accumulated in the leaves throughout the night.

One other experiment done in the artificial pools was a comparison of diurnal changes in malic aid concentration in submerged and emergent leaves. Submerged leaves had a 6-fold increase in malic acid overnight, whereas approximately adjacent emergent leaves showed less than a 2-fold increase. This pattern was the same whether expressed as a fraction of fresh weight or chlorophyll. Thus this pathway appears to be facultative and of lesser importance as the pools dry.

The data presented here are consistent with the hypothesis that CO_2 availability is greatly reduced during the day in these pools and that this puts a premium on CO_2-fixation at night in *Isoetes*. Recent gas exchange studies indicate that at the free CO_2 levels present at night in these pools, *Isoetes howellii* is capable of substantial *net* CO_2 uptake at night (Keeley and Bowes unpublished data). Further studies are needed to elaborate the relative contribution of dark and light CO_2 fixation.

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